

## RISES IN TITERS OF ANTIBODY TO HUMAN CORONAVIRUSES OC43 AND 229E IN SEATTLE FAMILIES DURING 1975-1979

ORTWIN W. SCHMIDT,<sup>1</sup> INEZ D. ALLAN,<sup>2</sup> MARION K. COONEY,<sup>3</sup> HJORDIS M. FOY,<sup>2</sup> AND  
JOHN P. FOX<sup>2</sup>

Schmidt, O. W. (Oklahoma College of Osteopathic Medicine and Surgery, Tulsa, OK 74107), I. D. Allan, M. K. Cooney, H. M. Foy, and J. P. Fox. Rises in titers of antibody to human coronaviruses OC43 and 229E in Seattle families during 1975-1979. *Am J Epidemiol* 1986;123:862-8.

Sequential serum specimens were obtained every four months during 1975-1979 from 44 children and adults of 10 Seattle families. The 419 specimens were tested for antibody to human coronaviruses OC43 and 229E by enzyme-linked immunosorbent assay (ELISA). Antibody titers were found to increase with age, and titers as well as frequency of rises were greater for OC43 than for 229E virus in all age groups. Significant antibody rises were most frequent in specimens bracketing the winter interval, but some also occurred in the spring-summer and summer-fall intervals. Concurrent significant antibody rises to OC43 virus in different members of the same family were observed in 15 instances, to 229E virus in seven instances, and to OC43 virus in some members and 229E virus in others in eight instances. Significant antibody rises to OC43 or 229E virus indicating reinfections were frequently observed throughout the three-year period but were always separated by at least two four-month intervals. Concurrent significant antibody rises to both 229E and OC43 viruses were seen only in three persons. Finally, the frequency of significant antibody rises in children, about one per person-year, was almost three times higher than in adults.

antibodies; coronavirus infections; respiratory tract infections

Human coronaviruses have been identified as etiologic agents of acute upper respiratory tract infections (1-5), and have been implicated in other diseases (6-14).

Received for publication March 1, 1985, and in final form July 22, 1985.

Abbreviations: ELISA, enzyme-linked immunosorbent assay.

<sup>1</sup> Department of Laboratory Medicine, School of Medicine, University of Washington, Seattle, WA.

<sup>2</sup> Department of Epidemiology, School of Public Health and Community Medicine, University of Washington, Seattle, WA.

<sup>3</sup> Department of Pathobiology, School of Public Health and Community Medicine, University of Washington, Seattle, WA.

Reprint requests to Dr. Ortwin W. Schmidt at current address: The Oklahoma College of Osteopathic Medicine and Surgery, Tulsa, OK 74107.

This study was supported by grants AI 18416 and AI 12269 from the National Institute of Allergy and Infectious Diseases, NIH.

Seroepidemiologic studies in the United States and other parts of the world have shown that antibodies to human coronaviruses OC43 and 229E are widespread in populations. Variations in the prevalence of antibodies appear to depend on several factors, including the population studied, the season when samples were taken, and the sensitivity of the serologic assay used. In a previous paper (15) we defined the parameters of an enzyme-linked immunosorbent assay (ELISA) for the measurement of human serum antibodies to specific antigens of 229E and OC43 viruses, the prototypes of the two accepted antigenic groups of human coronaviruses (1, 3, 16, 17). We also determined which antigens are primarily involved in the measurement of antibodies by ELISA and compared ELISA

results to results obtained with virus neutralization, complement fixation, and hemagglutination inhibition. In this report we describe the application of ELISA in seroepidemiologic studies of coronaviruses in 10 Seattle families under intensive continuing surveillance for influenzavirus infections during 1975-1979 (22).

## MATERIALS AND METHODS

### *Preparation of viral antigens*

The source of human coronavirus strains 229E and OC43 and their propagation have been described previously (18). Briefly, plaque purified virus strains were grown in human rhabdomyosarcoma cell cultures, purified by rate zonal and isopycnic centrifugation, and concentrated to 4 mg/ml of protein (19, 20). Whole virus antigens were prepared from purified virions by dissociation of concentrated virus samples in 1 per cent sodium dodecyl sulfate at 4 C for 10 minutes and subsequent dilution in coating buffer (0.1 M carbonate-bicarbonate, pH 9.6) immediately before coating of ELISA plates (15).

### *Enzyme-linked immunosorbent assay*

ELISA was carried out in Immulon M-129 microtiter plates with flat-bottomed wells, as previously described (15). All reagents were dispensed in aliquots of 110  $\mu$ l per well. Plates were coated with antigens (0.8  $\mu$ g of protein per well) diluted in carbonate-bicarbonate coating buffer (21) and serial 0.5  $\log_{10}$  serum dilutions were prepared directly in the antigen-coated plates in phosphate-buffered saline containing 0.2 per cent Tween 20 and 100  $\mu$ g/ml of gelatin. Affinity purified heavy chain-specific goat anti-human immunoglobulin G alkaline-phosphatase conjugate (Sigma Chemical Co., St. Louis, MO) was added at a dilution of 1:1,000. Para-nitrophenylphosphate (1 mg/ml) in 10 per cent diethanolamine buffer was used as substrate solution (21) and optical densities were determined with a "Titertek Multiskan" filter photometer (Flow Laboratories, McLean, VA) at 405

nm. Human sera devoid of antibody to human coronaviruses 229E and OC43 as determined by virus neutralization and radioimmune precipitation (15) were used as appropriate negative serum controls. Assays were carried out in duplicate and all specimens obtained from the same family were tested in the same run to avoid differences attributable to run-to-run variation.

Serum antibody titers were expressed as the reciprocal of the highest serum dilution which yielded optical densities of 0.35. These cutoff points were safely in the linear portion of the antibody titration curves of all sera tested and were significantly increased (at least two standard deviations) over nonspecific reactions (15). Antibody rises were expressed as ELISA ratios, which were defined as the ratios of maximum to minimum optical densities between sequential serum specimens at the same dilution. ELISA ratios of 1.8 or greater (i.e., a 1.8-fold increase in optical density) were accepted as indicating significant rises. These ratios were found to correlate with fourfold or greater increases in virus neutralizing and complement-fixing antibody titers in paired sera from 200 unrelated patients with respiratory disease (unpublished data).

### *Study population*

Of some 200 Seattle families entering into continuing observation for influenza-virus infections during 1975-1979, 10 were selected because each had at least one preschool child at entry into the study in fall 1975. The study population and methods of observation have been described in detail elsewhere (22). Nose-throat specimens were collected for culture biweekly and additional specimens were obtained when illness was observed. Blood specimens were collected three times yearly: late fall (October/November), spring (March/April), and late summer (July/August). A total of 419 blood specimens were examined. Of the 44 persons studied, nine were in the 0-4-year age group at entry, 11 in the 5-9-year

group, four in the 10–14-year group, and 20 were adults.

## RESULTS

### *Age-specific antibody titers to coronaviruses OC43 and 229E*

Only two children, both less than two years old, lacked antibody to 229E virus and one also lacked antibody to OC43 virus. All other persons had antibodies to both viruses. Titers ranged from 100 (reciprocal of endpoint serum dilution) in the very young to 60,000 in persons with a recent antibody rise. The 419 sera tested were distributed according to the age of the donor when blood samples were collected and the geometric mean titers for each age group were calculated. As seen in table 1, the geometric mean titers increased directly with age. Also, in each age group, the geometric mean titer for OC43 virus exceeded that for 229E virus, the relative difference decreasing with age from 2.8-fold (0–4 years) to 1.2-fold (adults).

### *Antibody rises in relation to age*

Examination of the 419 sera available revealed 92 significant antibody rises, 56 to OC43 virus and 36 to 229E virus. Overall, each study member experienced 0.7 significant antibody rises (to either OC43 or 229E virus) per year. However, as seen in table 2, the annual frequencies of rises to each

TABLE 1

*Geometric mean antibody titers to coronaviruses 229E and OC43\* in different age groups of Seattle families, 1975–1979*

Age group (years)	229E virus	OC43 virus
0–4	364.5	1,016.1
5–9	817.9	1,315.4
10–14	1,081.3	1,368.9
Adults	1,709.9	2,087.5

\* ELISA antibody titers were defined as the reciprocal of the highest serum dilution which yielded an optical density of 0.35 at 405 nm. Geometric mean titers were based on 419 sera grouped by age of donor when sampled. Antibody titers ranged from 100 to 32,000 for 229E virus and from 100 to 60,000 for OC43 virus.

TABLE 2

*Number of rises in ELISA antibody titers to human coronaviruses 229E and OC43 per person-year of observation,\* 1975–1979*

Ages (years)	Person years of observation	Rises per person-year	
		229E virus	OC43 virus
0–4	27.1	0.44	0.55
5–9	23.7	0.31	0.58
10–14	15.4	0.39	0.39
Adults	61.8	0.15	0.24
All	128.0	0.28	0.42

\* Significant antibody rises, defined as ELISA ratios of 1.8 or greater between two sequential serum specimens.

virus tended to decline with age. In all but the 10–14-year age group, antibody rises to OC43 virus were more frequent than were rises to 229E virus.

### *Temporal patterns of antibody rises*

Antibody rises to at least one virus (usually both) were recognized in all 10 sampling intervals (figure 1). With OC43 virus, the peak rises in each year occurred in the period between the October/November and March/April samplings and are reflected in the sera collected each spring. No similar pattern for 229E virus was observed.

### *Concurrent antibody rises*

As seen in figure 2, concurrent significant antibody rises to both viruses were observed in three children (one each in families E, I, and J). This number does not differ significantly from the number (five) expected on the basis of the observed frequencies of rises to each antigen.

Concurrent significant antibody rises to OC43 virus in at least two members of the same family were observed in 15 instances. With 229E virus there were seven such instances. Significant antibody rises to OC43 virus in some family members and to 229E virus in others were observed in eight instances.

### *Reinfection with human coronaviruses*

Primary infection, defined as seroconversion to either virus, was seen in only two of

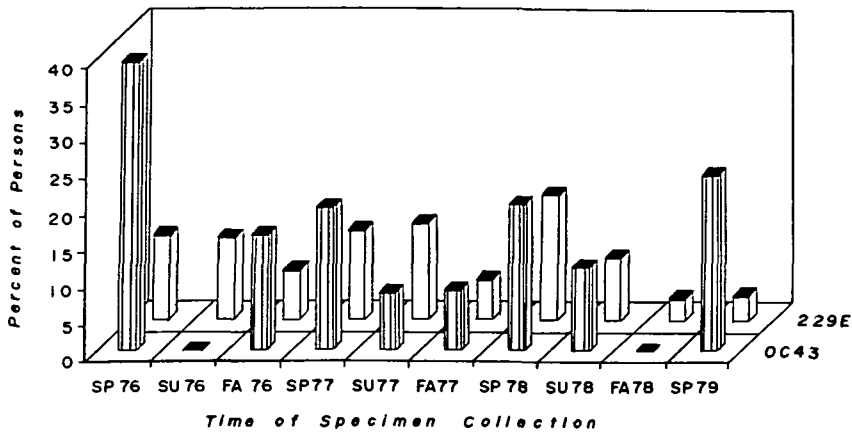


FIGURE 1. Rises in antibody titers to human coronaviruses OC43 and 229E in 10 Seattle families, 1975-1979. SP, SU, and FA denote times of blood specimen collection in spring (March/April), summer (July/August), and fall (October/November). Rises were assigned to the time of collection of the indicative specimen.

FAMILY MEMBERS	FAMILY	AGE	ANTIBODY RISES									
			SP 76	SU 76	FA 76	SP 77	SU 77	FA 77	SP 78	SU 78	FA 78	SP 79
A	M 34	2	●	▲	●	●			●	▲		●
		5	●						●			●
B	M 23	2	▲							●		
		10	▲							○		○
C	M 27	1			●	▲			●	▲		○
		2			○							●
D	M 29	1			●	▲			○	▲		●
		7			●	○			○			
E	M 29	1	●			▲				○		
		3	●			▲						
F	M 33	2	●	▲	●	○	▲		○			
		6	●		○		▲		●			
G	M 27	5	●	▲		○			▲			○
		7	●			○			●			○
H	M 28	5	▲	▲		●	▲		▲			▲
		7	▲			○	▲		▲			▲
I	M 29	3	▲		▲		●	○		▲	▲	●
		7	▲				●			▲		○
J	M 31	2	●		▲	●			▲	○		○
		5	●			●						
	F 36	8	●									
		12	●									
		13	●									
		13	●									

FIGURE 2. Rises in antibody titers to human coronaviruses OC43 and 229E in 10 Seattle families, 1975-1979. Significant antibody rises (ELISA ratios of 1.8 or greater) between two sequential serum specimens are denoted by closed symbols for OC43 (●) and 229E (▲) viruses, respectively. Lesser increases in antibody levels (open symbols) are shown for comparison, and were not used in calculations. SP, SU, FA denote spring (March/April), summer (July/August) and fall (October/November), respectively. Families (A-J) and age of persons in years at entry into the program in fall 1975 are shown. Mothers (M) and fathers (F) are identified. —denotes specimen was not available for testing.

the three children less than two years of age. In one child (family C), seroconversion to OC43 virus was observed between August and November 1976, followed by seroconversion to 229E virus between November 1976 and March 1977. The other child (family D) had antibody to OC43 virus initially but expressed seroconversion to 229E virus between November 1976 and March 1977. Apparent reinfections with coronaviruses as indicated by repeated significant antibody rises to the same virus were seen in 21 instances with OC43 virus and in eight with 229E virus. In each instance, these were separated by at least eight months. Two significant antibody rises in succession were observed in 14 persons, in each instance to different viruses.

#### *Antibody rises in relation to illness*

Because of the four-month intervals between collection of blood specimens, it was not possible to relate specific illnesses to observed rises in antibody titers. However, it was noted that 36 per cent of significant antibody rises in adults and 40 per cent in children had occurred during intervals in which no respiratory or gastrointestinal illness was reported. The two children with primary coronavirus infections had experienced respiratory illnesses unrelated to isolation of or detectable rises in antibody to other viruses. Further, OC43 viral antigens were detected in nose-throat specimens from one of the two children by ELISA (unpublished data).

#### DISCUSSION

The observed high prevalence of antibodies in Seattle families (prevalences from 30 to 100 per cent have been reported for other populations) probably reflects both the increased sensitivity of the assay and the age of the study population since, in other studies, children less than two years of age, also often seronegative, were relatively more numerous. We also found antibody levels to be directly related to age as has been reported by others (23-36), presumably reflecting the cumulated effects of

repeated infections. Antibody levels, after initial increases with age, remained generally stable within individuals except when presumed reinfection caused a sharp but temporary increase in titers with usual return to baseline levels within four to eight months.

The intervals between bleedings did not coincide with the conventionally defined four seasons since the original intent was to bracket influenza seasons. However, marked temporal differences in the frequency of significant antibody rises to OC43 virus were seen with the highest frequencies being observed in the predominantly winter interval between the October/November and March/April samplings. No such pattern was evident for 229E virus.

The combined infection rate, approaching one per person-year, was similar to that reported for an adult population in London, England (32). However, it is probable that the rates reported in this study underestimate the true frequency of infections. Our definition of a significant antibody rise, ELISA ratios of 1.8 or greater, was rather conservative. This ratio was accepted based on its correlation with fourfold or greater rises in virus neutralizing and complement-fixing antibody titers in paired sera from 200 unrelated persons. ELISA ratios of between 1.5 and 1.8 also probably represented increases in specific antibody levels since many of these correlated with fourfold rises in virus neutralizing antibody titers and, in this study, 18 of 32 occurred in family clusters of two or more or coincidentally with 1.8 ratios in other family members. Our findings are in agreement with the present knowledge of the epidemiology of human coronaviruses but they also suggest that coronavirus infections in families are more frequent than has been thought.

Based on their independent frequencies, concurrent antibody rises in the same person to both 229E and OC43 viruses should occur only rarely. Both our observation of three such instances as well as findings reported by others (32, 33) conform with

this expectation. Given the length of the periods between bleedings (four months), the concurrent rises could reflect either consecutive or concurrent infections. On the other hand, concurrent antibody rises to each virus in different family members were seen frequently and in all seasons. Obviously, the two viruses circulated in the population simultaneously as well as independently, as has been reported by others (2, 23, 26, 27, 32, 34, 36-41).

Antibody rise to OC43 virus in one season was followed frequently by a rise to 229E virus or vice versa in the same person in the next season, but recurrent rises to the same virus were always separated by at least eight months. These results suggest that infection with one virus may convey short-term immunity to homologous reinfection.

Primary infections were observed in only two children. Both children had reported illness episodes. On the other hand, reinfections as indicated by significant antibody rises to either virus were common but their association with specific illnesses was not possible. Indeed, about 40 per cent of all significant antibody rises occurred without reported temporally related illness. We have presumed that these rises reflected reinfections with viruses related to one or the other prototype but it remains possible that at least some reflect chronic infections accompanied by antibody rises when host defenses weakened. Prolonged coronavirus antigen detection in throat and nose specimens from persons in London, England, over periods of up to two months has been reported (42). Preliminary studies of antigen detection in nasopharyngeal specimens from persons in the Seattle study have yielded similar results (unpublished data). If these findings can be confirmed, our present understanding of the epidemiology of human coronaviruses would be considerably expanded.

#### REFERENCES

1. Hamre D, Procknow JJ. A new virus isolated from the human respiratory tract. *Proc Soc Exp Biol Med* 1966;121:190-3.
2. Kapikian AZ, James HD Jr, Kelly SJ, et al. Iso-

- lation from man of "avian infectious bronchitis-like" viruses (coronaviruses) similar to 229E virus, with some epidemiological observations. *J Infect Dis* 1969;119:282-90.
3. McIntosh K, Becker WB, Chanock RM. Growth in suckling mouse brain of "IBV-like" virus from patients with upper respiratory tract disease. *Proc Natl Acad Sci USA* 1967;58:2268-73.
4. McIntosh K, Dees JH, Becker WB, et al. Recovery in organ cultures of novel viruses from patients with respiratory disease. *Proc Natl Acad Sci USA* 1967;57:933-40.
5. Tyrrell DAJ, Bynoe MC. Cultivation of a novel type of common cold virus in organ cultures. *Br Med J* 1965;1:1467-70.
6. Arnold W, Klein M, Wang JB, et al. Coronavirus-associated antibodies in nasopharyngeal carcinoma and infectious mononucleosis. *Arch Otorhinolaryngol* 1983;232:165-77.
7. Burks JS, DeVald BL, Jankovsky LD, et al. Two coronaviruses isolated from central nervous system tissue of two multiple sclerosis patients. *Science* 1980;209:933-4.
8. Caul EO, Egglestone SI. Further studies on human enteric coronaviruses. *Arch Virol* 1977;54:107-17.
9. Chany C, Moscovici O, Lebon P, et al. Association of coronavirus infection with neonatal necrotizing enterocolitis. *Pediatrics* 1982;69:209-14.
10. Henigst W. Vorkommen von Antikörpern gegen Coronavirus (OC43) in der gesunden Bevölkerung und bei Respirationstraktkranken. *Zbl Bakt Hyg I. Abt Orig* 1974;A229:150-8.
11. Marshall JA, Birch CJ, Williamson HG, et al. Coronavirus-like particles and other agents in the faeces of children in Efate, Vanuata. *J Trop Med Hyg* 1982;85:213-15.
12. Riski H, Hovi T. Coronavirus infection of man associated with diseases other than common cold. *J Med Virol* 1980;6:259-65.
13. Salmi A, Ziola B, Hovi T, et al. Antibodies to coronaviruses OC43 and 229E in multiple sclerosis patients. *Neurology* 1982;32:292-5.
14. Vaucher YE, Ray CG, Minnich LL, et al. Pleomorphic, enveloped, virus-like particles associated with gastrointestinal illness in neonates. *J Infect Dis* 1982;145:27-36.
15. Schmidt OW. Antigenic characterization of human coronaviruses 229E and OC43 by enzyme-linked immunosorbent assay. *J Clin Microbiol* 1984;20:175-80.
16. Bradburne AF, Somerset BA. Coronavirus antibody titres in sera of healthy adults and experimentally infected volunteers. *J Hyg* 1972;70:235-44.
17. MacNaughton MR, Madge MH, Ree SE. Two antigenic groups of human coronaviruses detected by using enzyme-linked immunosorbent assay. *Infect Immun* 1981;33:734-7.
18. Schmidt OW, Cooney MK, Kenny GE. Plaque assay and improved yield of human coronaviruses in a human rhabdomyosarcoma cell line. *J Clin Microbiol* 1979;9:722-8.
19. Schmidt OW, Kenny GE. Immunogenicity and antigenicity of human coronaviruses 229E and OC43. *Infect Immun* 1981;32:1000-6.
20. Schmidt OW, Kenny GE. Polypeptides and functions of antigens from human coronaviruses 229E

- and OC43. *Infect Immun* 1982;35:515-22.
21. Voller A, Bidwell DE, Bartlett A. The enzyme-linked immunosorbent assay. Guernsey, England: Dynatek Europe, Borough House, 1979:7-124.
  22. Fox JP, Hall CE, Cooney MK, et al. Influenzavirus infections in Seattle families, 1975-1979. I. Study design, methods and the occurrence of infections by time and age. *Am J Epidemiol* 1982;116:212-27.
  23. Candeias JAN, Carvalho RPS, Antonacio F. Seroepidemiologic study of coronavirus infection in Brazilian children and civilian adults. *Rev Inst Med Trop São Paulo* 1972;14:121-5.
  24. Gerna G, Achilli G, Cattaneo E, et al. Determination of coronavirus 229E antibody by an immune-adherence hemagglutination method. *J Med Virol* 1978;2:215-23.
  25. Gerna G, Cattaneo E, Cereda P, et al. Seroepidemiologic studies of human coronavirus OC43 infections in Italy. *Sieroterapico Milanese* 1978; 57:535-42.
  26. Gerna G, Cereda PM, Ravello MG, et al. A rapid microneutralization test for antibody determination and serodiagnosis of human coronavirus infections. *Microbiologica* 1979;2:331-44.
  27. Hasony HJ, Macnaughton MR. Prevalence of human coronavirus antibody in the population of southern Iraq. *J Med Virol* 1982;9:209-16.
  28. Hovi T, Kainulainen H, Ziola B, et al. OC43 strain-related coronavirus antibodies in different age groups. *J Med Virol* 1979;3:313-20.
  29. Kaye HS, Dowdle WR. Seroepidemiologic survey of coronavirus (strain 229E) infections in a population of children. *Am J Epidemiol* 1975;101:238-44.
  30. Kaye HS, Marsh HB, Dowdle WR. Seroepidemiologic survey of coronavirus (strain OC43) related infections in a children's population. *Am J Epidemiol* 1971;94:43-9.
  31. Lehel F, Madar Z, Toth I. Antibodies to coronavirus OC43 strain in the population of north-eastern Hungary. *Acta Microbiologica Hungarica* 1983;30:163-6.
  32. MacNaughton MR. Occurrence and frequency of coronavirus infections in humans as determined by enzyme-linked immunosorbent assay. *Infect Immun* 1982;38:419-23.
  33. Mathur A, Arora KL, Rajvanshi S, et al. Coronavirus in respiratory infection. *Indian J Med Res* 1982;75:323-8.
  34. McIntosh K, Kapikian AZ, Turner HC, et al. Seroepidemiologic studies of coronavirus infection in adults and children. *Am J Epidemiol* 1970; 91:585-92.
  35. Sarateanu DE, Ehrengut W, Fofana Y. Influenza and coronavirus antibodies in the newborns and their mothers in Mali (1980). *Tropenmed Parasit* 1980;31:399-401.
  36. Zakstelskaya LY, Sheboldov AV, Vasilieva VJ, et al. Occurrence of antibody to coronavirus in sera of people living in the USSR. *Voprosy Virusologii* 1972;17:161-5.
  37. Hendley JO, Fishburne HB, Gwaltney JM Jr. Coronavirus infections in working adults: Eight year study with 229E and OC43. *Am Rev Resp Dis* 1972;105:805-11.
  38. Monto AS, Lim SK. The Tecumseh study of respiratory illness: VI. Frequency of and relationship between outbreaks of coronavirus infection. *J Infect Dis* 1974;129:271-6.
  39. Wenzel RP, Hendley JO, Davies JA, et al. Coronavirus infections in military recruits: Three-year study with coronavirus strains OC43 and 229E. *Am Rev Resp Dis* 1974;107:621-4.
  40. Hamre D, Beem M. Virologic studies of acute respiratory disease in young adults. V. Coronavirus 229E infections during six years of surveillance. *Am J Epidemiol* 1972;96:94-106.
  41. Cavallaro JJ, Monto AS. Community-wide outbreak of infection with a 229E-like coronavirus in Tecumseh, Michigan. *J Infect Dis* 1970;122:272-9.
  42. Macnaughton MR, Flowers D, Isaacs D. Diagnosis of human coronavirus infection in children using enzyme-linked immunosorbent assay. *J Med Virol* 1983;11:319-25.